Opposing Effects of CD70 Costimulation during Acute and Chronic Lymphocytic Choriomeningitis Virus Infection of Mice

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T cell costimulation is important for T cell activation. The CD27/CD70 pathway contributes to effector and memory T cell development and is involved in T cell and B cell activation. CD27/CD70 is known for having opposing roles during different models of antigenic challenges. During primary T cell responses to influenza virus infection or during tumor challenges, CD27/CD70 costimulation has a positive role on T cell responses. However, during some chronic infections, constitutive triggering of this signaling pathway has a negative role on T cell responses. It is currently unclear what specific characteristic of an antigen determines the outcome of CD27/CD70 costimulation. We investigated the effect of a transient CD70 blockade during an acute or a chronic lymphocytic choriomeningitis virus (LCMV) infection in mice. Blockade of this pathway during acute LCMV infection (Armstrong strain) resulted in delayed T cell responses and decreased CD127 (interleukin-7 receptor [IL-7R]α chain) conversion. Upregulation of CD127 is an important event in T cell differentiation that heralds the passage of an effector T cell to a long-lived memory T cell. In contrast to the reduced CD8 T cell responses after CD70 blockade during acute infection, CD70 blockade during chronic LCMV infection resulted in increased CD8 T cell responses. Our data show the dual roles of this costimulatory pathway in acute versus persistent antigen challenge. Our findings suggest that antigen persistence may determine the effect of CD27/CD70 signaling on CD8 T cell responses. Tailored triggering or blockade of this costimulatory pathway may be important in vaccination regimens against acute or chronic pathogens.
CD70 pathway may have different roles, depending on the persistence or perhaps the type of the antigen. In order to resolve these observations, we took advantage of the LCMV system. This model of infection has both an acute and a chronic infection model with identical T cell epitope specificities. We show here that after acute infection with LCMV Armstrong, CD27/CD70 signaling contributes to the induction of an efficient T cell response since blockade of this pathway results in delayed T cell responses. This was evidenced by reduced absolute numbers of antigen-specific CD8 T cells at the peak of the antiviral T cell response and by slower CD127 conversion, a marker for memory commitment (21). Conversely, after chronic infection with the chronic LCMV clone 13 (Cl-13) strain, CD27/CD70 costimulation negatively regulates T cell responses since blockade of this pathway results in increased CD8 T cell responses after 3 weeks postinfection. Our data suggest that context-dependent regimens to manipulate the CD27/CD70 pathway may be of interest in the design of T cell-based vaccines, depending on the persistence of the pathogen.

MATERIALS AND METHODS

Mice and infections. Six- to 8-week-old, female C57BL/6 mice were purchased from the Jackson Laboratories (Bar Harbor, ME). Mice were infected intraperitoneally (i.p.) with LCMV Armstrong (2 × 10⁶ PFU) or intravenously (i.v.) with Cl-13 (2 × 10⁶ PFU) via lateral tail vein injection.

Virus titration. Titration of LCMV was performed on Vero cell monolayers plated on six-well plates as described previously (2). Vero cells were grown on minimal essential medium (MEM) supplemented with 10% fetal calf serum (FCS), 1% L-glutamine, and penicillin-streptomycin (PenStrep). Samples were diluted in 1% FCS-Dulbecco's modified Eagle's medium (DMEM) and aliquoted on top of cell monolayers. Plates were incubated for 1 h, with rocking every 10 min. Wells were then overlaid with a 1:1 mixture of 1% agaro and 2× 199 medium. After 4 days, wells were overlaid with a 1:1 mixture of 1% agaro and 2× 199 medium containing 1:50 neutral red. Plaques were counted the following day.

In vivo antibody blockade. A total of 300 μg of rat anti-mouse CD70 (FR70) or rat IgG2b isotype control (diluted in phosphate-buffered saline [PBS]) was administrated i.p. every 3 days starting on day 0 (days 0, 3, 6, 9, and 12 after LCMV infection). This regimen was similar to that used by Matter et al. (24), in which mice were treated twice a week. FR70 monoclonal antibody was prepared as described previously (26).

Intracellular cytokine and surface staining. Intracellular cytokine and surface staining were performed as described previously (37). LCMV peptide stimulations were performed at 37°C for 5 h in a CO₂ incubator in the presence of GolgiPlug and GolgiStop (BD Biosciences). LCMV peptides were obtained from the Emory Microchemical Facility (Atlanta, GA). All monoclonal antibodies for flow cytometry were purchased from BD Biosciences (San Jose, CA). Stained cells were acquired using a FACSCanto flow cytometer (Becton Dickinson) and analyzed using FlowJo (Treestar).

Antibody titration. Serum LCMV-specific IgG titers were titrated by a solid-phase direct enzyme-linked immunosorbent assay (ELISA) as previously described (2), using horseradish peroxidase (HRP)-conjugated antibodies against mouse IgG on ELISA 96-well microplates. Serum from Armstrong-infected day 40 mouse (positive control) or naive mouse (negative control) was tested at the same time.

Statistical analysis. GraphPad (Prism) software was used for statistical analysis. A Mann-Whitney test was used for calculating statistical significance.

RESULTS

Role of CD27/CD70 signaling on CD8 T cell responses during acute viral infection. We first decided to analyze the effect of blocking CD27/CD70 signaling during an acute viral infection. We injected C57BL/6J mice i.p. with CD70 blocking antibodies on the day of LCMV Armstrong infection and again every 3 days, for a total of five times, prior to the analysis of acute cellular responses at days 7 and 15 (Fig. 1A). The frequencies (Fig. 1B) and absolute numbers (Fig. 1C) of LCMV-specific CD8 T cell responses at day 7 postinfection were reduced compared to control levels. The percentages of tumor necrosis factor alpha (TNF-α)-expressing CD8⁺ T cells were also lower in anti-CD70-treated mice, as shown on the representative plots, especially at early time points (day 7). This result suggested a positive role for CD70 costimulation in primary responses. Although the percentages (Fig. 1D) of CD8 T cells that were LCMV specific were similar by day 15 postinfection, there was still a modest but consistent reduction in their absolute numbers (Fig. 1E) after CD70 blockade.

Memory conversion after CD70 blockade during acute viral infection. In addition to reduced primary CD8 T cell responses after CD70 blockade, virus-specific (H-2D b GP33-41-positive) CD8 T cells displayed a delayed CD127 conversion by day 15 postinfection, as evidenced by reduced per cell expression of CD127, and diminished percentages of CD127⁺ CD8 T cells (Fig. 1F). These data suggested defects not only in the magnitude of the virus-specific T cell response but also in memory conversion. Polyfunctionality of antigen-specific CD8 T cells was also compromised, especially at the peak of the antiviral CD8 T cell response. The absolute numbers of gamma interferon (IFN-γ) and TNF-α-coexpressing CD8 T cells at this time were also reduced after CD70 blockade, but by day 15, cytokine-coexpressing cells were just slightly decreased (Fig. 2).

Role of CD27/CD70 signaling on CD4 T cell responses during acute LCMV infection. In contrast to the reduced percentages of virus-specific CD8 T cells after CD70 blockade during acute LCMV infection, the percentages of virus-specific CD4 cells (expressing IFN-γ after peptide GP61-80 stimulation) were not different from those of control mice (see Fig. S1 in the supplemental material). Nevertheless, there was a modest reduction in their absolute numbers at days 7 and 15 postinfection. This is because of a reduced spleen cell count in anti-CD70-treated mice. Normally, acute LCMV Armstrong infection is readily cleared within 8 days (36). Reduction in the numbers of LCMV-specific T cells after CD70/CD27 blockade during acute infection resulted in a slight delay in virus control in the spleen and serum at day 5, but the differences were not statistically significant (see Fig. S2). At day 60 postinfection, T cell responses were similar to those of control mice and showed a normal PD-1-negative and CD127-high phenotype, suggesting stabilization of responses several weeks after the interruption of CD70/CD27 blockade (see Fig. S3). It is possible that longer CD70 blockade regimens may result in more extended functional defects, and we are currently investigating this.

Effect of CD27/CD70 signaling on antibody responses during acute LCMV infection. The effect of anti-CD70 monoclonal antibody blockade on LCMV-specific IgG antibody responses was opposite to the effect on CD8 T cells. Even though the CD8 T cell response was delayed after CD70 blockade, the LCMV-specific IgG antibody titers were slightly enhanced at day 15 after LCMV Armstrong infection (Fig. 3). This suggested a positive effect of CD70 costimulation on CD8 T cell responses but a negative role on antibody responses.
Role of CD27/CD70 signaling on CD8 T cell responses during chronic LCMV infection. In order to evaluate the effects of CD70 blockade in a chronic LCMV infection, we took advantage of the LCMV Cl-13 strain. For this, we injected C57BL/6J mice with CD70 blocking antibodies on the day of LCMV Cl-13 infection and again every 3 days, for a total of five times, and analyzed immune responses at days 7 and 21 postinfection (Fig. 4A). In contrast to acute LCMV Armstrong infection, CD70 blockade during chronic LCMV Cl-13 infection resulted in no change in the frequencies (Fig. 4B) and numbers (Fig. 4C) of LCMV-specific CD8 T cell responses at day 7 postinfection. However, during the later stage of chronic infection (day 21), even though the frequencies (Fig. 4D) of antigen-specific CD8 T cell responses were similar between CD70 blockade and control mice, there was an almost 2-fold increase in the absolute number (Fig. 4E) of virus-specific CD8 T cells after CD70 blockade. This clearly suggested a negative role for CD27/CD70 during chronic infection. Although absolute numbers of virus-specific CD8 T cells were increased in the CD70 blockade group, their phenotype was similar to that of control mice, showing the expected CD127- and PD-1-exhausted phenotype (Fig. 5).

Role of CD27/CD70 signaling on CD4 T cell responses during chronic LCMV infection. At day 7 postinfection, there was no difference in the percentages or the absolute numbers of virus-specific CD4 T cells. However, by day 21 postinfection, even though the percentages of virus-specific CD4 T cells were similar, the absolute numbers were increased in anti-CD70-treated mice (see Fig. S4 in the supplemental material). This is in sharp contrast to the situation in acutely infected mice, in which there is a reduction in LCMV-specific CD4 T cells (GP61-80 peptide specific).

Effect of CD27/CD70 signaling on antibody responses and viral control during chronic LCMV infection. Transient CD70 blockade had a moderately positive effect on antibody responses at day 21 postinfection during chronic LCMV infection (Fig. 6A). This pattern of increased antibody responses after CD70 blockade was similar to that during acute infection. Short-term blockade of CD70 signaling during chronic LCMV infection resulted in a modest and transient reduction in viral loads that was noticeable...
only at day 15 postinfection but returned to normal values after interruption of treatment (Fig. 6B).

**DISCUSSION**

T cell costimulation is an important event after TCR triggering (11), as it provides a requirement for T cell activation. Signals from CD27 and the TCR mediate CD8 T cell clonal expansion independently of interleukin-2 (IL-2) and contribute to promoting T cell survival (7). CD70 is usually expressed on activated antigen-presenting cells (APCs), but both CD27 and CD70 can be expressed on T cells (18, 19, 24). Therefore, this pathway has been suggested to be involved not only in DC-T cell interactions but also in the T cell-T cell network (23). Expression of CD70 on immune cells is tightly regulated. CD70 is upregulated at 1 to 2 weeks after infection and gradually decreases over time after an antigenic challenge, highlighting the importance of its proper regulation on immune cells (24). The duration and cell type involved in CD27/CD70 costimulation also determine the outcome of immune responses. For example, mice that overexpress CD70 constitutively on B cells display increased effector T cell differentiation and decreased numbers of B cells, suggesting opposite effects of constitutive CD27/CD70 signaling on T and B cell responses (4).

Previous reports have shown that CD27/CD70 signaling enhances antitumor (3, 10, 13, 17, 25) and primary influenza virus-specific T cell responses (1, 5, 12, 17, 34). Consistent with these reports, we found delayed primary response kinetics after blockade of the CD27/CD70 pathway after acute Armstrong LCMV infection. At day 7 postinfection, the peak of the antiviral T cell response, there were fewer absolute numbers of antigen-specific T cells by intracellular cytokine staining. Also, percentages and absolute numbers of CD8 T cells that coexpressed IFN-γ and TNF-α were reduced after CD70 blockade at the peak of the primary response. This suggested functional defects after CD70 blockade during an acute viral infection. Viral clearance kinetics seemed to be slightly delayed in the anti-CD70-treated group by day 5, but acute Armstrong infection was cleared in both groups. This suggested that even if the numbers of T cells that recognize LCMV Armstrong get cur-
tailed 3-fold, this reduced fraction of the response is more than enough to completely clear this virus.

After CD70 blockade during acute LCMV infection, CD127 conversion was also impaired. CD127, the IL-7 receptor α chain, is a bona fide marker for memory T cells as those T cells that persist after the contraction phase following the peak of the T cell response are usually CD127^{hi}. These CD127^{hi} cells are known to become long-lived cells and respond to secondary challenges (16, 21). Our results with the acute LCMV model were in agreement with previous reports using either CD70 antibody blockade or CD27^{−/−} mice. These earlier studies have shown that CD27/CD70 signaling is necessary for the generation and maintenance of primary CD8 T cell responses. Primary clonal T cell expansion after infection with influenza virus is impaired in CD27^{−/−} mice independent of CD28 and IL-2 (17), demonstrating the importance of the CD27/CD70 pathway during an acute infection. In addition, this pathway facilitates IL-2-independent CD8 T cell clonal expansion without effector differentiation (7), suggesting an important role in the initial expansion of a primary response.

The aforementioned studies showed a positive role for CD27/CD70 costimulation on T cell responses. Abrogation of CD27/CD70 signaling either with receptor knockout mice or antibody-mediated blockade results in reduced numbers of antigen-specific T cell responses during primary acute responses. However, there is also evidence of an opposing role of CD27/CD70 costimulation during chronic infection, suggesting a negative effect of constitutive CD27/CD70 signaling on immune responses (24, 33, 34). Constitutive expression of CD70 on T cells induces a T cell differentiation pathway that is similar to that observed during chronic infections such as HIV, including upregulation of PD-1 and downregulation of CD127 (34). Mice that overexpress CD70 on B cells succumb to lethal immunodeficiency after 6 to 8 months due to overt immune activation and spontaneous conversion of naïve T cells into effector memory cells (33). These results, together with those in the aforementioned models, suggested context-dependent outcomes of CD27/CD70 costimulation. In order to consolidate these previous data, we decided to use the LCMV system, which has both an acute and a chronic model of infection. This helped us to determine whether the persistence or the nature of an antigen plays a role in the outcome of CD70 costimula-
LCMV Armstrong and Cl-13 strains differ in only two point mutations in the polymerase and the receptor binding genes (29, 30). These subtle differences enable the Cl-13 strain to persist and cause progressive T cell exhaustion (37, 39). Thus, the nature of the antigen is virtually identical, with all T cell epitopes unchanged, and the only significant difference between these two LCMV strains is their persistence within the host.

As a comparative study, we decided to analyze the effect of blocking CD27/CD70 interactions during chronic LCMV infection (comparing effects seen on acute LCMV infection). A previous report has demonstrated a negative role of CD70 signaling during chronic viral infection, showing that the absence of signaling through CD27 during a chronic LCMV Docile viral challenge results in elimination of chronic viral infection (24).

In our studies, we performed only a transient CD70 antibody blockade, and we noticed increased absolute numbers of antigen-specific T cells in the polymerase and the receptor binding genes (29, 30). These subtle differences enable the Cl-13 strain to persist and cause progressive T cell exhaustion (37, 39). Thus, the nature of the antigen is virtually identical, with all T cell epitopes unchanged, and the only significant difference between these two LCMV strains is their persistence within the host.

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Consistent with the results from Matter et al. (24), we found some increase in B cell responses after CD70 blockade during chronic LCMV infection (blockade in these reports was performed from day 4 to day 35 postinfection). We did not notice significant reductions in viral titers after 12 days of anti-CD70 antibody treatment. The studies from Matter et al. (24) were performed using CD27−/− mice as well as with longer blocking antibody regimens. Our more short-term blockade approach may account for the reduced and merely transient effect on viral titers and the more modest increase in antibody responses that we observed during CD70 blockade after either chronic or acute infection. Our data comparing acute Armstrong infection with the more virulent chronic Cl-13 are also in agreement with a previous study that shows that virulence determines costimulation receptor usage (28). In this recent report from Michael Croft’s laboratory, the authors show that weakly replicating pathogens preferentially induce the CD28 costimula-

FIG. 5. No change in the phenotype of virus-specific CD8 T cells after CD70 blockade during chronic viral infection. First column shows percentage of CD8+ T cells that are H-2Db GP33-41 tetramer positive. Second and third columns are gated on tetramer-positive cells and show expression of the memory marker CD127 and the inhibitory receptor PD-1. Data are from spleens in two separate experiments (each, n = 6).

FIG. 6. Virus-specific antibody responses and viral control after CD70 blockade during chronic viral infection. ELISA was performed for detection of LCMV-specific IgG titers from serum at day 21 postinfection. Sera from an Armstrong-infected mouse at day 40 postinfection (memory) and from a naïve mouse were included as positive and negative controls, respectively. Data are from one representative experiment.
tory pathway but that the OX-40 and CD27 pathways (which identify effector CD8 T cells) give rise to long-lived memory cells. Nat. Immunol. 5:11001–11006.


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